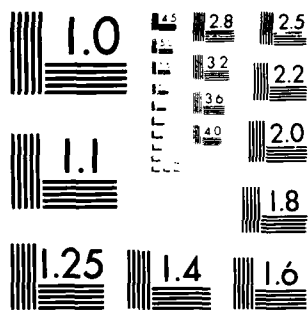


RESEARCH AND DEVELOPMENT OF WOUND DRESSING IN  
MAXILLOFACIAL TRAUMA(U) BIOTEK INC WOBURN MA  
D L WILLIAMS ET AL. 23 JUN 82 2110-3 DAMD17-81-C-1204

NI

F/G 6/12

END  
DATE  
FILMED  
1 -- 85  
DTC



MICROCOPY RESOLUTION TEST CHART  
 NATIONAL BUREAU OF STANDARDS-1963-A

APPROVED AT GOVERNMENT EXPENSE

12

AD-A148 548

RESEARCH AND DEVELOPMENT OF WOUND  
DRESSING IN MAXILLOFACIAL TRAUMA

DISTRIBUTION STATEMENT A

Approved for public release  
Distribution Unlimited

**BIOTEK, Inc.**

84 12 05 016

21-C Olympia Avenue, Woburn, MA 01801 Tel: (617) 938-0938

DTIC  
ELECTE  
DEC 14 1984

S

D

D

DTIC

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
AI	

(12)

AD \_\_\_\_\_

Report No. 2110-3

RESEARCH AND DEVELOPMENT OF WOUND  
DRESSING IN MAXILLOFACIAL TRAUMA

ANNUAL SUMMARY REPORT

David L. Williams, Ph.D.  
James H. Kerrigan, M.S.  
David E. Creedon, B.S.  
Shirley A. Odell, B.S.

June 23, 1982

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-81-C-1204

**DTIC**  
**ELECTE**  
**DEC 14 1984**  
**S D**

BIOTEK, Inc.  
21-C Olympia Avenue  
Woburn, MA 01801

Approved for public release; distribution unlimited

The findings in this report are not to be construed  
as an official Department of the Army position unless  
so designated by other authorized documents.

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER	
	DA148548		
4. TITLE (and Subtitle)		5. TYPE OF REPORT & PERIOD COVERED	
Research and Development of Wound Dressing in Maxillofacial Trauma		Annual 10/1/81-5/31/82	
		6. PERFORMING ORG. REPORT NUMBER	
		2110-3	
7. AUTHOR(s)		8. CONTRACT OR GRANT NUMBER(s)	
David L. Williams, Ph.D. Shirley A. Odell, B.S. James H. Kerrigan, M.S. David E. Creeden, B.S.		DAMD17-81-C- <del>1105</del> 1204	
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	
BIOTEK, Inc. 21-C Olympia Avenue Woburn, MA 01801		62775A.35162775A825.AA.053	
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE	
U.S. Army Medical Research and Development Command Fort Detrick, Frederick, MD 21701-5012		June 23, 1982	
		13. NUMBER OF PAGES	
		44	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report)	
		unclassified	
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report)			
Approved for public release; distribution unlimited			
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)			
18. SUPPLEMENTARY NOTES			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)			
Biological and Medical Sciences - Pharmacology Wound healing Hemostatics Polymers Local anesthetics Encapsulating Polylactide Antiinfectives and antibacterials Powder (Particles) Fibers			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)			
<p>Because of the natural molecular weight distribution of poly-L(-)lactide polymerization, batches can be blended with viscosities as disparate as R.S.V. = <math>1.2 \pm 0.6</math> dl/g. Fabrics are prepared by spraying a solution of polymer and drug. Powders are prepared by grinding a cast film of polymer and drug with dry ice. Materials were characterized by drug content and timed release, and by SEM and EDAX (energy dispersive analysis of X-rays).</p> <p>(over)</p>			

unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Povidone-iodine (PVP-I<sub>2</sub>) fabrics were prepared from 20% to 75% drug. With less drug the fabric was more fibrous, but drug release was less. A 40% drug fabric was chosen for further testing. EDAX of these fabrics showed less iodine on the surface than in the total fabric. PVP-I<sub>2</sub> powders released active iodine rapidly from a 20% drug composite.

Anesthetic fabrics were prepared with benzocaine, bupivacaine-HCl, etidocaine-HCl, and lidocaine base, hydrochloride, and hydrosulfate. Generally the base form of the anesthetics deliver drug in a more continuous manner than the salt forms, and etidocaine-HCl release is more continuous than bupivacaine-HCl release. In these *in vitro* tests, about 10% of the drug is released in 1 hour and 25% is released within 6 hours; about 60% of the drug remains in the fabric after 24 hours of release testing. These conclusions are based on 20% lidocaine (base), 20% benzocaine, and 15% etidocaine-HCl fabric samples.

Anesthetic powders were prepared with similar materials. Larger particles show more sustained release. Powders release drug more rapidly than the corresponding fabrics, but similar drugs release continuously.

Sample storage studies indicate that benzocaine samples cannot be stored for one year at 40°C, unless the sample is hermetically sealed. All other tested samples were stable.

Biological testing included Avitene testing by controlled bleeding of a dermatome-inflicted wound, and hemoglobin analysis of the dressing. PVP-I<sub>2</sub> fabric was tested for bacteriostatic effectiveness using a cell culture of Bacillus subtilis in tryptic soy broth. The fabric and solution are equally effective versus three successive inoculations of the bacteria.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

## FOREWARD

In conducting the research described in this report, the investigator(s) adhere to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of (DHEW Publication No. (NIH) 78-23, Revised 1978).

## TABLE OF CONTENTS

	<u>Page</u>
Report Documentation Page	i
Foreward	iii
Table of Contents	iv
List of Figures	1
List of Tables	2
I. Summary	3
II. Accomplishments	5
A. Physicochemical Testing	5
1. Release of Drug from Homogeneous Systems	5
2. Material Preparation	5
a. Polymer Availability and Measurements	5
b. Selection and Preparation of Drugs	9
c. Preparation of Fabrics	10
d. Preparation of Powders	10
3. Methods Development and Results	13
a. Surface Drug Measurement Method	13
b. <u>In Vitro</u> Drug Release	14
1) Testing of Fabrics	14
2) Testing of Powders	16
4. Analysis of Results	16
a. Povidone-Iodine Release from Fabric	19
b. PVP·I <sub>2</sub> Release from Powder	22
c. Anesthetic Release from Fabrics	22
d. Anesthetic Release from Powders	22
5. Bupivacaine Comparisons	27
6. General Conclusions on Anesthetic Release	32
7. Storage Stability Testing	32
B. Biological Testing	36
1. Povidone-Iodine Evaluation	36
2. Avitene Evaluation	40
III. References	43
Distribution List	44



LIST OF FIGURES

	<u>Page</u>
Figure 1 Gel Permeation Chromatograms	8
Figure 2 SEMs of PVP-I <sub>2</sub> -Polylactide Fabric Showing Structural Difference as Drug Concentration is Increased	21
Figure 3 SEMs of PVP-I <sub>2</sub> -Polylactide Powder of 20% Drug (212-300 microns) Showing structure of Fractured Surface	23
Figure 4 SEM, Sample, and Release Data for 20% Lidocaine (Base) Fabric	24
Figure 5 SEM, Sample, and Release Data for 20% Benzocaine Fabric	25
Figure 6 SEM, Sample, and Release Data for 15% Etidocaine-HCl Fabric	26
Figure 7 SEM, Sample, and Release Data for 10% Lidocaine (Base) Powder of 300-425 microns	28
Figure 8 SEM, Sample, and Release Data for 15% Etidocaine-HCl Powder of 300-425 microns	29
Figure 9 Bupivacaine-HCl Fabric and Powder at 20% Drug Loading	30
Figure 10 Comparison of Etidocaine-HCl and Bupivacaine-HCl at 20% Loading in Fabric and Powder 150-212 microns	31

LIST OF TABLES

		<u>Page</u>
Table 1	Effect of Viscosity (R.S.V.) on Polymer Blends	7
Table 2	5% Lidocaine.HCl Size Distribution After Three Passes through Straub Grinder	11
Table 3	Size Distribution of Prepared Drug Powders	12
Table 4	Fabric Data as Percent Release	15
Table 5	Powder Data as Percent Release	17
Table 6	Samples Stored for Future Analysis	34
Table 7	Assay and Drug Release of Powders After One Year of Storage	35
Table 8	Inhibition of <u>Bacillus Subtilis</u> Growth by Pure PVP·I <sub>2</sub>	38
Table 9	<u>B. Subtilis</u> Inhibition by PVP·I <sub>2</sub> in Fabric	39

## I. SUMMARY

Because of the natural molecular weight distribution of poly-L(-)lactide polymerization, batches can be blended with viscosities as disparate as  $R.S.V. = 1.2 \pm 0.6$  dl/g. Fabrics are prepared by spraying a solution of polymer and drug. Powders are prepared by grinding a cast film of polymer and drug with dry ice. Materials were characterized by drug content and timed release, and by SEM and EDAX (energy dispersive analysis of X-rays).

Povidone-iodine ( $PVP \cdot I_2$ ) fabrics were prepared from 20% to 75% drug. With less drug the fabric was more fibrous, but drug release was less. A 40% drug fabric was chosen for further testing. EDAX of these fabrics showed less iodine on the surface than in the total fabric.  $PVP \cdot I_2$  powders released active iodine rapidly from a 20% drug composite.

Anesthetic fabrics were prepared with benzocaine, bupivacaine·HCl, etidocaine·HCl, and lidocaine base, hydrochloride, and hydrosulfate. Generally the base form of the anesthetics deliver drug in a more continuous manner than the salt forms, and etidocaine·HCl release is more continuous than bupivacaine·HCl release. In these in vitro tests, about 10% of the drug is released in 1 hour and 25% is released within 6 hours; about 60% of the drug remains in the fabric after 24 hours of release testing. These conclusions are based on 20% lidocaine (base), 20% benzocaine, and 15% etidocaine·HCl fabric samples.

Anesthetic powders were prepared with similar materials. Larger particles show more sustained release. Powders release drug more rapidly than the corresponding fabrics, but similar drugs release continuously.

Sample storage studies indicate that benzocaine samples cannot be stored for one year at 40°C, unless the sample is hermetically sealed. All other tested samples were stable.

Biological testing included Avitene testing by controlled bleeding of a dermatome-inflicted wound, and hemoglobin analysis of the dressing. PVP-I<sub>2</sub> fabric was tested for bacteriostatic effectiveness using a cell culture of Bacillus subtilis in tryptic soy broth. The fabric and solution are equally effective versus three successive inoculations of the bacteria.

## II. ACCOMPLISHMENTS

### A. Physicochemical Testing

#### 1. Release of Drug from Homogeneous Systems

Drug release from a homogeneous composite can be expected to release drug more rapidly at the start of the diffusion period, since the drug will initially be released from the surface of the powder or fiber. However, in microcapsules, a constant release is possible because a reservoir of drug exists in the center of the capsule (Contract DAMD17-81-C-1195).

In considering the optimum drug release from a homogeneous system the Higuchi model of release applies; the rate of release decreases with the square root of time (Baker and Lonsdale, 1974). If 20% is released in one hour, then it follows that 49% will be released within 6 hours. This is a reasonable goal of this project. Fortunately most of a drug needs to be released in the earlier stages of wound covering. Also if less drug is delivered in a continuous manner, then more material may be applied to the wound.

#### 2. Material Preparation

##### a. Polymer Availability and Measurements

In the proposal we indicated that the use of poly-L(-)lactide of R.S.V. = 1.2 dl/g from batches of R.S.V. between 1.0 and 1.5 dl/g was unnecessarily restrictive. Since the polymerization process produces a wide range of molecular weights, a less restrictive R.S.V. range is warranted.

At the start of the contract period we had approximately 1,450 grams of the polymer blend (R.S.V. = 1.19 dl/g). This material was prepared for Contract Nos. DAMD17-80-C-0093 and DAMD17-80-C-0110 and can be used on this contract and Contract No. DAMD17-81-C-1195. Based on the broad molecular weight distribution of the previously prepared polymer, we now believe that a broad range of viscosities (R.S.V.) can be blended, without affecting the final molecular weight distribution. This broad molecular weight distribution was also found to be natural for the process. The theoretical polydispersity of a completed condensation polymerization reaction is 2.0 (Flory, 1953). The polydispersity ( $M_w/M_n$ ) of our polymer is 2.33.

Polymer 5-6-27 of R.S.V.  $\sim$  0.72 dl/g and Polymer 5-6-25 of R.S.V.  $\sim$  2.23 dl/g were, therefore, precipitated with isopropanol:methylene chloride (3:1). These polymers were dried, ground, and stored in the freezer. At this time the viscosities were R.S.V. = 2.04 dl/g for Polymer 5-6-25 and R.S.V. = 0.73 dl/g for Polymer 5-6-27. Weights are 390 grams and 375 grams, respectively.

To prove that these batches could be blended, a sample of 0.73 dl/g polymer (5-6-27) and 2.04 dl/g polymer (5-6-25) were analyzed by gel permeation chromatography. Also a combination of 33% of the low and 67% of the high molecular weight polymer was analysed. The results are summarized in Table 1 and compared with the initial polymer analysis of Contract No. DAMD 17-80-C-0110. At equal concentrations of the two polymers (0.73 and 2.04 dl/g) the chromatogram overlap is 48% (Figure 1). The combination chromatogram does not show a bimodal distribution but does show a tailing at high molecular weights.

Table 1  
EFFECT OF VISCOSITY (R.S.V.) ON POLYMER BLENDS

<u>R.S.V.</u>	<u>Polymer</u>	<u>MWw</u>	<u>MWn</u>	<u>MWw/MWn</u>
0.73	5-6-27	29,880	17,590	1.70
2.04	5-6-25	87,840	52,020	1.69
1.20*	Blend of Above	57,420	22,860	2.51
1.19	Previous Contract Blend	45,700	21,500	2.14

\* calculated R.S.V.

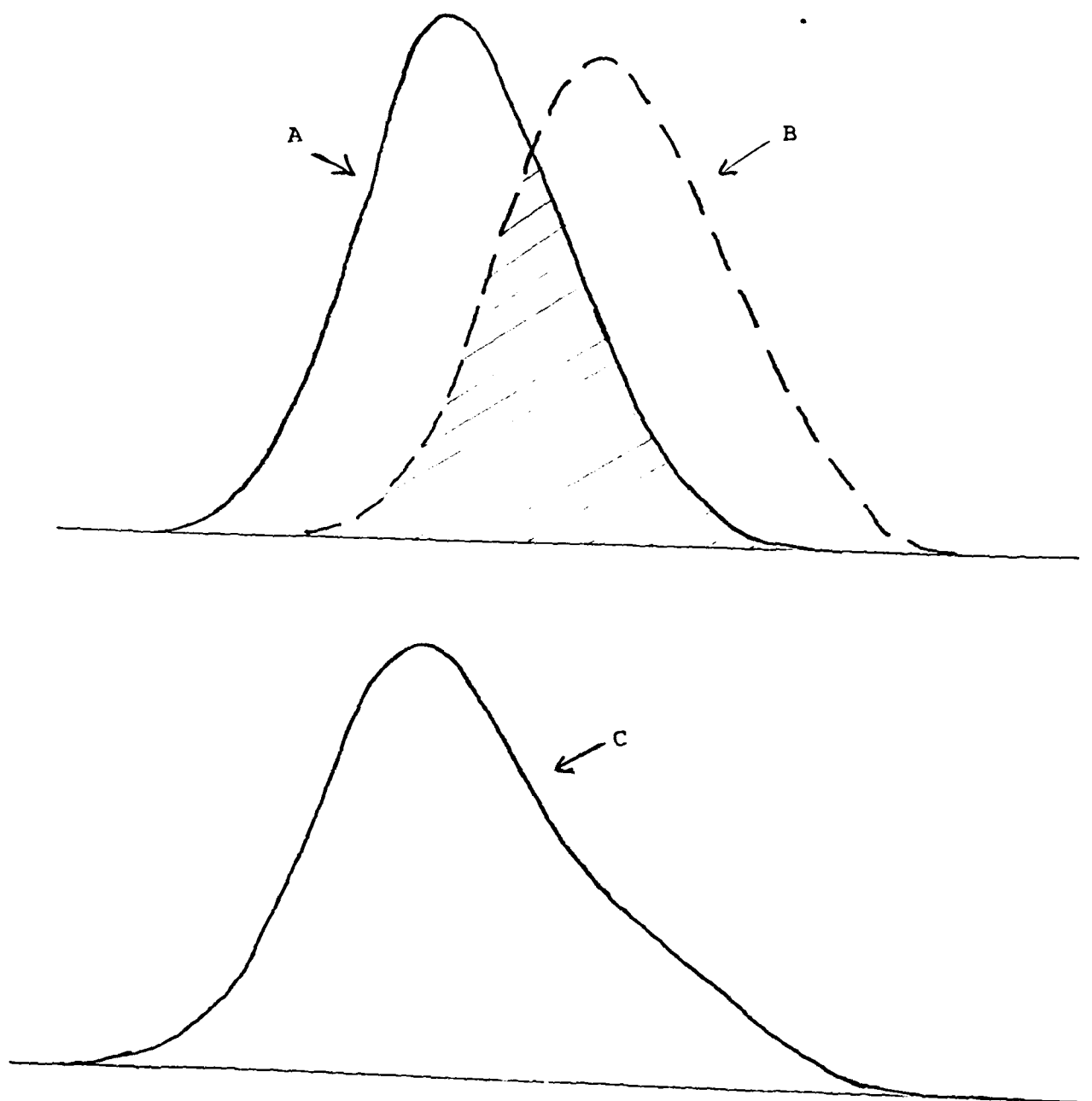


Figure 1 Gel Permeation Chromatograms of  
A. 5-6-27, R.S.V. = 0.73 Pd = 1.70  
B. 5-6-25, R.S.V. = 2.04 Pd = 1.69  
C. 2 of 5-6-27 and 1 of 5-6-25  
R.S.V. = 1.2 Pd = 2.51



Thus we can probably blend this wide a viscosity range without affecting the polymer coating and drug release properties of the polymer blend. When needed, these polymers will be blended (dissolved in  $\text{CH}_2\text{Cl}_2$ ) in a 1:3 ratio to give an R.S.V. of  $\sim 1.20$  dl/g. Several additional polymer batches are presently available in the 0.6-0.8 dl/g range.

#### b. Selection and Preparation of Drugs

Lidocaine salts can be prepared by precipitation of the less soluble salt in water or by addition of the appropriate acid to the base form of the drug. Best results were obtained by dissolving the lidocaine (base) in ethanol and adding concentrated sulfuric or phosphoric acid solutions (aqueous). The precipitates were then washed with ethanol. Lidocaine sulfate has a melting point of  $\sim 215^\circ\text{C}$  and is slightly soluble in water. Lidocaine phosphate has a melting point of  $\sim 180^\circ\text{C}$ , but it is very soluble in water. Thus the lidocaine sulfate is the salt of choice for powder and fabric preparation.

Various iodine complexes were originally proposed, since slow release of a polymeric iodine complex might not diffuse through polylactide. Low molecular weight polyvinylpyrrolidone (PVP) and  $\text{PVP}\cdot\text{I}_2$  materials were requested from various suppliers. The original  $\text{PVP}\cdot\text{I}_2$  was prepared with a high molecular weight PVP (K30). For excretion by the kidneys, a lower molecular weight is advised for internal use, such as open wounds (K17, Schlezing, Mahinka, and Levin, 1979). However, lower molecular weight  $\text{PVP}\cdot\text{I}_2$  is less stable to  $\text{I}_2$  loss (e.g. after 14 days at  $52^\circ\text{C}$  there is 6% loss with K30, and 12% loss with K17). Using K12 PVP ( $n\sim 23$ ) the loss exceeds

the DOD requirement. However, a sample of K12 PVP has been requested and received (BASF). GAF is attempting to prepare a K8 PVP.

Various monomeric pyrrolidone·I<sub>2</sub> complexes were described by Schenck, Simak and Haedicke (1979). An attempt to prepare a methylpyrrolidone-iodine adduct by their method was unsuccessful.

#### c. Preparation of Fabrics

The spraying conditions were varied from those previously described. After several trial runs to prepare a 15% lidocaine fiber mat, a more concentrated solution was prepared in methylene chloride. The final fabrics were prepared by spraying a 10% polymer solution, with added drug, in a methylene chloride solution onto a gauze fabric. The spray was continuous, using a small arc to cover the 15 cm target. The target was in the hood which removed small fibers from the laboratory area.

#### d. Preparation of Powders

As in the previous program, powders were prepared by grinding a film of material, which had been formed by casting from a methylene chloride solution. Ten percent methanol was added to solubilize bupivacaine·HCl. Lidocaine·HCl (5%) samples were prepared by Dr. Roy at Mass. College of Pharmacy using the Mikro-Pulverizier hammer mill with a 20 mesh classifier screen. A similar sample was ground at BIOTEK using a Straub grinder. Both samples were ground with dry ice added to the feed material. The Straub grinder generated coarse particles. These particles had a brownish tinge and contained iron particles from the grinding head. The decision was made at this point to continue to send samples to the Mass. College of Pharmacy for grinding.

The sieve analysis of the particles is shown in Tables 2 and 3.

TABLE 2  
5% Lidocaine·HCl  
Size Distribution After Three Passes  
Through Straub Grinder

<u>Size Fraction</u> <u>(<math>\mu</math>m)</u>	<u>%</u>
> 600	78.9
425-600	14.1
300-425	4.3
212-300	1.7
150-212	0.7
106-150	0.3
75-106	-
38-75	-
< 38	-

TABLE 3  
 SIZE DISTRIBUTION OF PREPARED DRUG FOWDERS  
 (Data is % of Total Milled Sample)

Size (um)	5% Lidocaine-HCl	10% Lidocaine (Base)	10% Bupivacaine-HCl	20% Bupivacaine-HCl	30% Etidocaine-HCl	30% Benzocaine
> 600	6.2	0.0	1.1	2.1	2.1	0.0
600-425	37.4	8.4	30.1	25.8	32.5	8.5
425-300	13.8	33.8	21.3	22.0	17.0	46.8
300-212	8.5	20.2	13.5	14.1	14.5	24.9
212-150	7.6	12.8	10.8	10.9	12.2	9.5
150-106	10.7	12.0	10.2	10.9	10.5	6.3
106-74	11.4	7.7	7.3	8.1	7.0	2.5
74-35	3.9	4.8	5.1	5.8	4.0	1.6
< 38	0.5	0.3	0.5	0.4	0.3	0.0

### 3. Methods Development and Results

#### a. Surface Drug Measurement Method

The analysis of exposed drug on the surface of non-woven fabric and powders might explain some of the differences observed between different forms of these drug-polymer homogeneous systems. Thus the fabric is formed from evaporation of solvent, and a high concentration of polymer on the surface might explain the slow release of fabric versus powders (e.g., 20% bupivacaine). Most of the exposed powder surfaces are generated along fracture planes during milling at dry ice temperature. There also may be a precipitous increase of surface drug as the percent of drug is increased in the formulation.

The concentration of heavy elements can be quantified on the surface by energy dispersive analysis of X-rays (EDAX). Thus the chlorine atoms of the anesthetic hydrochlorides and the iodine atoms of povidone iodine can be analyzed and correlated to the drug concentration.

The analysis was performed with an EDAX 707 and Amray 1000 SEM. Samples were coated with a thin layer of gold-palladium, since graphite coating might cause drug decomposition and sublimation of iodine or hydrogen chloride. A 20 Kev beam was used for analysis. This would penetrate about 2 microns into the sample, assuming a sample density of  $1.5 \text{ g/cm}^3$ . The radius of the analysed spot on the surface is approximately one micron.

Pure samples of povidone iodine and bupivacaine hydrochloride had iodine and chlorine peaks of 41 and 68 counts/second, respectively. Percent drug on the surface of fabrics and powders were correlated by ratios to these values.

Since no chlorine was found in the PVP·I<sub>2</sub> sample, there is no methylene chloride interference in the anesthetic hydrochloride analysis. No tin could be found in the polymer by this method.

b. In Vitro Drug Release

Analysis of the data on the preceeding program was hampered by the fragmented reporting of the in vitro release data (from laboratory notebooks to reports). Therefore, master sheets were developed for data entry, and this data was then re-organized in terms of type of drug and percent loading. Assays were performed in methylene chloride. This also includes assays of residual drug from the spent particles. Hence calibration curves have been generated for each drug in aqueous and methylene chloride solvents. Bupivacaine and etidocaine samples are acidified and the diffusion sample discarded. The amount of drug lost is included in the calculation.

1) Testing of Fabrics

Twenty two different fabrics have been prepared and the release data are shown in Table 4. Choices of concentrations of drugs were chosen based on the results of the previous contract. Bupivacaine·HCl had not been tested previously, on this program.

TABLE 4  
FABRIC DATA AS PERCENT RELEASE

Organized by Drug

Drug	%	% Release in Hours					*Plus Residual
		1	2	4	6	24	%
Benzocaine	10	4	6	10	13	34	
	15	3	5	11	16	30	100
	20	8	13	20	25	44	
	20	7	11	19	25	43	90
	30	12	14	18	20	31	96
Bupivacaine·HCl	10	7	8	8	9	9	94
	15	6	10	11	12	12	98
	20	8	10	10	11	11	99
	30	19	23	26	27	31	105
	50	86	87	92	92	92	101
Etidocaine·HCl	10	9	16	20	21	29	
	10	5	8	20	21	26	97
	15	13	19	25	30	41	93
	16	7	14	24	32	44	104
	20	28	38	47	52	65	
Lidocaine·HCl	10	33	38	39	41	45	
	15	32	37	42	44	52	94
	20	25	26	24	22	26	
Lidocaine (base)	10	0	0	1	1	11	
	15	1	2	6	8	17	91
	15	2	5	7	10	23	108
	20	36	53	67	78	96	
	20	10	16	22	26	38	104
Lidocaine·H <sub>2</sub> SO <sub>4</sub>	20	58	61	61	62	62	
PVP·I <sub>2</sub> (BASF <sup>2</sup> 17/12)	10	0	0	0	0	0	
	20	0	0	0	0	0	
	**20(11)	0	0	0	0	0	106
	**30(19)	34	39	44	44	53	112
	**40(26)	72	85	95	100	114	131
	50	94	103	105	105	105	106
	50	87	100	106	106	115	117
	75	95	101	109	109	109	111
	75	37	53	62	70	92	100

\* Also indicates that samples prepared/tested during this contract year

\*\* Average of duplicate samples with and without 0.002% sodium lauryl sulfate. Values in parentheses are assays in CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH.

A representative section of fabric was selected and carefully removed from the gauze backing. These samples were weighed and used for assay and drug release studies. Lidocaine·H<sub>2</sub>SO<sub>4</sub> was prepared and fabric was prepared using a polymer-drug solution in 30% methanol-70% methylene chloride. Since an assay and residual analysis was not readily accomplished with lidocaine·H<sub>2</sub>SO<sub>4</sub> fabric, the calculation from absorbance data was based on an estimate of its equivalence as lidocaine (base).

## 2) Testing of Powders

In this program, drug release is being studied on the 75-106, 150-212, and 300-425 micron particles. All size fractions that are used for diffusion are independently assayed for the percent of drug. Selected powder samples of the previously prepared powders were re-sieved to generate large particle samples for drug release studies. Twenty-four powder samples were tested during this contract, as shown in Table 5.

In all cases, as theory predicts, the larger particles release their drugs more slowly. Also lower concentrations of drug in polymer release relative percentages of drug more slowly.

## 4. Analysis of Results

In this section the release of PVP·I<sub>2</sub> and anesthetics from fabrics and powders are described in more detail.



TABLE 5  
POWDER DATA PERCENT RELEASE  
(Organized by Drug)

Drug	% Drug**	Size ( $\mu$ m)	% Release in Hours					* Plus Residual
			1	2	4	6	24	
Benzocaine	10	300-425	6	7	9	9	13	
		150-212	22	23	26	27	33	
		74-106	33	33	36	37	43	
	15(18)	300-425	14	17	21	24	34	83
	(13)	150-212	32	39	43	47	60	104
	(17)	75-106	41	46	50	52	60	90
	20(21)	300-425	19	25	31	34	48	86
	(19)	212-300	34	44	51	56	66	98
	20	212-300	41	45	48	51	59	
		150-212	32	35	37	40	48	
		74-106	32	36	41	44	52	
Bupivacaine-HCl	10(10)	300-425	88	88	91	89	91	104
	(10)	105-212	94	95	96	94	96	110
	(10)	75-106	95	97	98	98	99	109
	20(16)	300-425	86	94	97	95	97	106
	(17)	105-212	94	94	96	94	95	105
	(17)	75-106	91	91	94	92	93	106
Etidocaine-HCl	10	300-425	15	24	33	37	53	
		150-212	24	37	46	48	55	
		74-106	28	38	44	45	51	
	15(12)	300-425	10	20	29	33	47	86
	(14)	150-212	38	43	48	51	59	95
	(13)	75-106	57	60	60	62	65	123
	20	425-600	20	26	39	48	69	
		300-425	24	38	44	52	61	87
		212-300	44	-	59	-	75	
	(22)	150-212	56	56	56	-	56	
		150-212	30	46	48	59	67	86
		75-106	73	-	76	-	76	
Lidocaine-HCl	5(6)	300-425	25	32	39	41	49	
	(7)	150-212	66	69	72	73	67	
	(7)	74-106	74	75	71	72	66	
	10	300-425	61	68	70	73	74	
		150-212	78	81				
		74-106	80	81				
	20(22)	300-425	64	68	72	73	74	86
		250-300	66	76	78	81	85	
	(21)	150-212	74	78	81	81	81	94
		150-212	75	78	77	78	79	
		75-106	69	74	73	73	71	

TABLE 5 (Cont.)  
POWDER DATA PERCENT RELEASE

(Organized by Drug)

<u>Drug</u>	<u>% Drug**</u>	<u>Size (<math>\mu</math>m)</u>	<u>% Release in Hours</u>					<u>* Plus Residual</u>
			<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>24</u>	
Lidocaine (base)	10(14)	300-425	18	22	28	30	41	
	(18)	150-212	43	48	53	56	63	
	(16)	75-106	55	59	63	64	68	
	20	300-425	61	71	83	91	103	
		150-212	100	111	119	122	123	
		75-106	107	112	116	118	121	
PVP-I <sub>2</sub> (Napp)	20	300-425	15	15	15	17	29	75
		150-212	33	37	37	43	49	62
		74-106	58	64	64	64	64	68
	(BASF)	300-425	72	72	76	79	79	109
	(17/12)	150-212	89	89	89	89	89	104
		74-106	104	104	104	104	104	110

\* Also indicates samples prepared and tested this year

\*\* Values in parenthesis are assay values

#### a. Povidone-Iodine Release from Fabric

The initial povidone-iodine fabric was tested for drug release from both sewn and heat-sealed bags. After 24 hours of release, the bags were opened and examined. Some sections of the bags contained clear (spent) polymer-PVP. Other areas of the bags were dark blue (iodine-starch product). In the center of this dark section was an area of orange material which turned dark blue when it was wetted with the release medium (KI-starch-buffer). This orange section appeared to be non-wetted fabric. This observation was probably independent of the type of bag, although the sewn bag has fewer crevices for bubble entrapment. The non-wetted fabric was found in a sewn diffusion bag with the largest quantity of fabric. This slow wetting phenomenon leads to a slow release of drug from the non-woven fabric. However, it is very dependent on the thickness of fabric and wetting ability of the diffusion medium. In this one test with 100 mg of fabric in the 2 x 2 cm bag (approximate) the drug release was encouraging:

<u>Time (hours)</u>	<u>Release (%)</u>
1	37
2	53
4	62
6	70
24	92

The sample was 75% drug and 25% polymer. Hence the fabric was very weak, but might be usable. However, with about 75 mg of fabric approximately 90% of the drug was released in one hour (Table 4).

These results are significantly different from the total lack of iodine release obtained in the previous contract with 10 and 20% drug.

Samples were then prepared with 20, 30, and 40% povidone-iodine in polylactide. Duplicate samples were tested, with and without 0.002% sodium lauryl sulfate as a wetting agent. Since there was no obvious effect of the surfactant, the results are averaged in Table 5. The results fall in line with the previous samples. However, there is little indication of a continuous release of active iodine at 20 and 30% drug. There appears to be a continuous release of drug from the 40% fabric. There is also a problem when comparing the assay procedure in aqueous and non-aqueous media. This gives an unreasonably low assay and residual value with respect to the aqueous release data.

Scanning electron micrographs have been obtained for many of these samples, courtesy of Colonel Mader at USAIDR. In Figure 2 is shown the PVP·I<sub>2</sub> fabrics at 20 and 50% drug loading. Excellent fabric can be formed with 20% PVP·I<sub>2</sub>, but the 50% material is much less fibrous. This is also evident from the scanning electron micrograph. The 40% drug fabric is intermediate in fibrous structure.

EDAX analysis of povidone-iodine fabric of 20 and 50% drug showed less iodine than expected. Nodules and spheres in the 50% fabric gave values of 25% surface iodine, and a thin fiber contained only 17% surface iodine. The 20% fabric sample yielded 16% surface iodine in fiber areas and about 12% in nodular areas. Perhaps surface iodine is lost in the process of sample preparation and analysis. However, the analysis does not show a clear trend which can be correlated with in vitro drug release data.

20% PVP·I<sub>2</sub>50% PVP·I<sub>2</sub>

Figure 2 SEMs of PVP·I<sub>2</sub>—poly(lactide) Fabric  
Showing Structural Difference as  
Drug Concentration is Increased

#### b. PVP·I<sub>2</sub> Release from Powder

Powders of PVP·I<sub>2</sub> at 20% loading are shown for comparison in Figure 3. The powders, which release faster than the fabrics, appear to have a much higher surface area than the corresponding fabrics. There also appears to be drug segregation on these fractured powder surfaces.

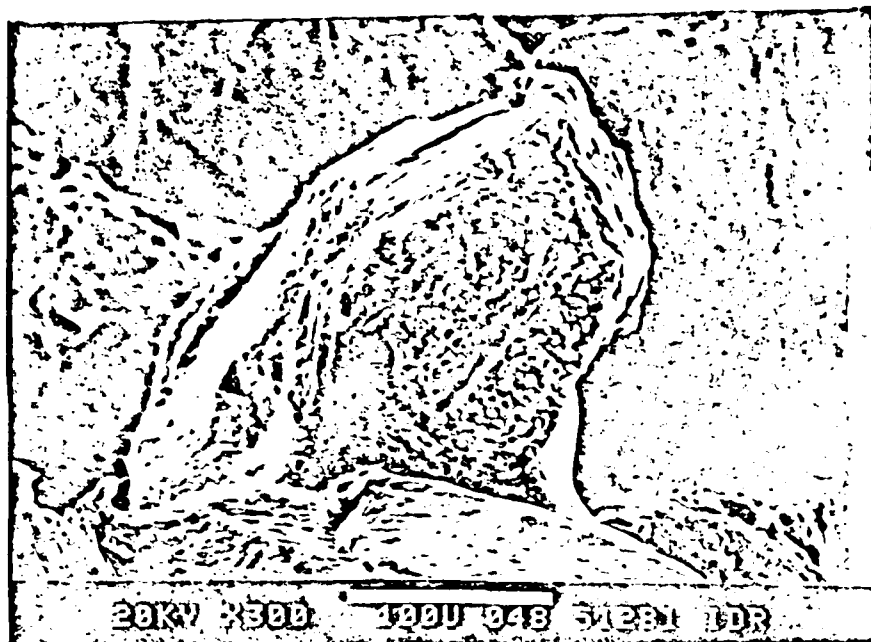
#### c. Anesthetic Release from Fabrics

Fabrics containing anesthetics appear to wet more completely than do those with PVP·I<sub>2</sub>. However, the wetting is more difficult to ascertain because there is no color change during the wetting and release processes.

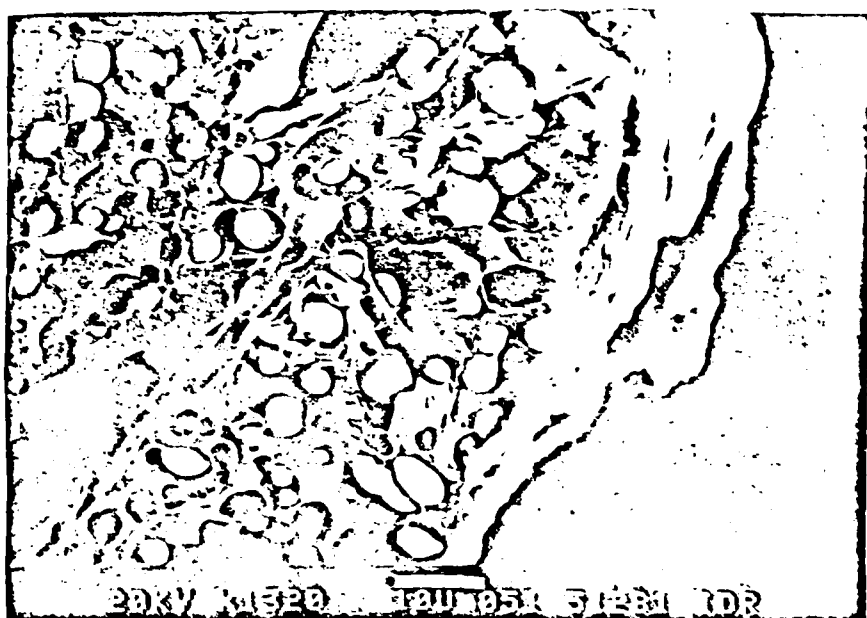
Anesthetic release from fabrics is generally similar to previous release from similar systems (Contract No. DAMD17-80-C-0093). Lidocaine·HCl releases 1/4 to 1/3 of the drug in one hour and very little thereafter. Lidocaine (base) releases in a more continuous manner (about 20% drug may be optimal). Benzocaine, an amine base, also releases in a continuous manner (20% or more may be optimal). Bupivacaine·HCl releases very little drug, and most of this is within the first hour. Etidocaine·HCl, at 15-20% loading, releases drug in a possibly useful manner. SEM, samples and drug release data are shown in Figures 4, 5, and 6 for some of the better anesthetic samples (i.e., 20% lidocaine·base, 20% benzocaine, and 15% etidocaine·HCl).

#### d. Anesthetic Release from Powders

The complete wetting of powders has been assumed. However, we have no unified theory on why some samples release drug more rapidly than other samples. Because there is a consistency of release which follows the diffusion limiting



x300



x1320

Figure 3 SEMs of PVP-I<sub>2</sub>-Polylactide Powder of 20% Drug (212-300 microns) Showing Structure of Fractured Surface

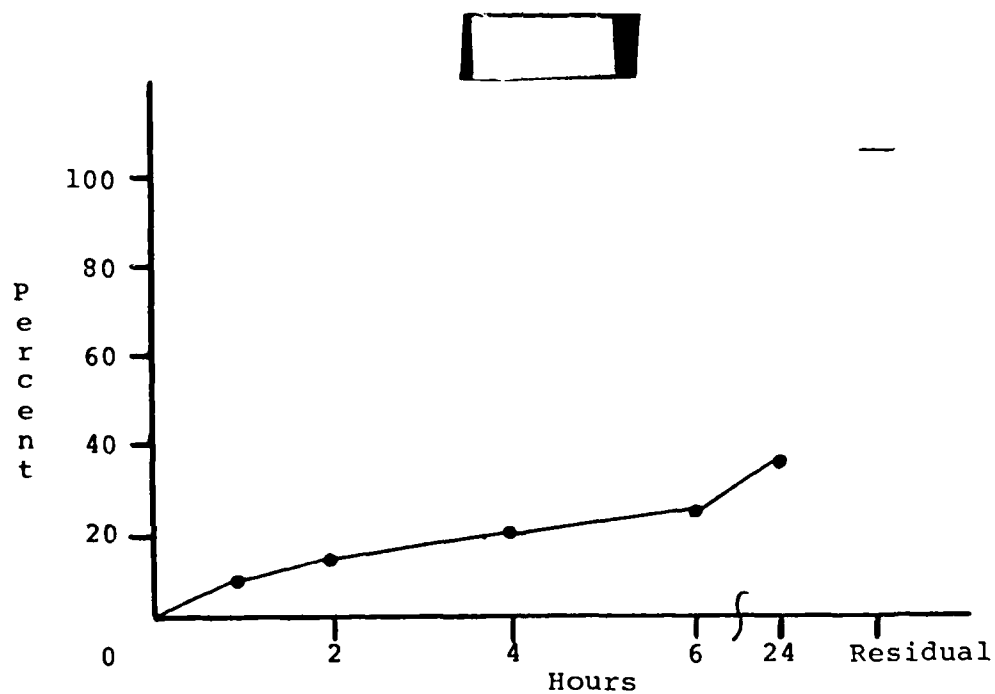
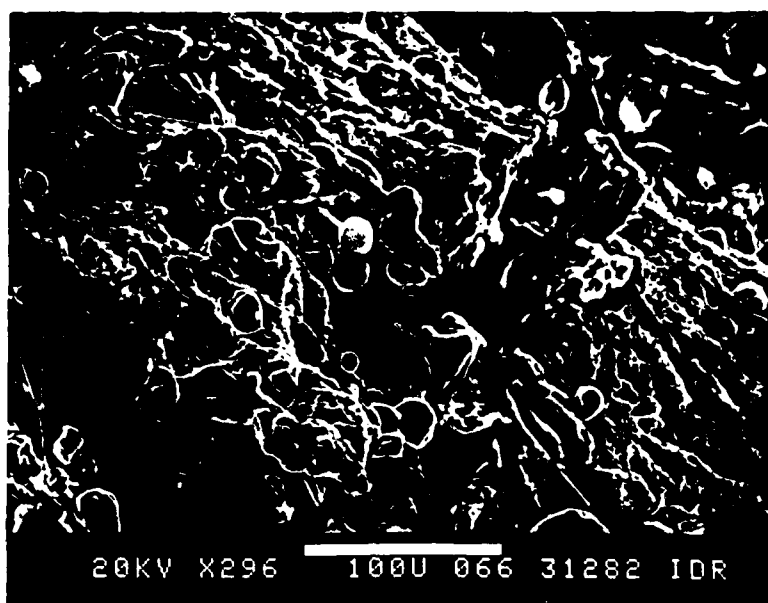


Figure 4 SEM, Sample and Release Data for  
20% Lidocaine (Base) Fabric



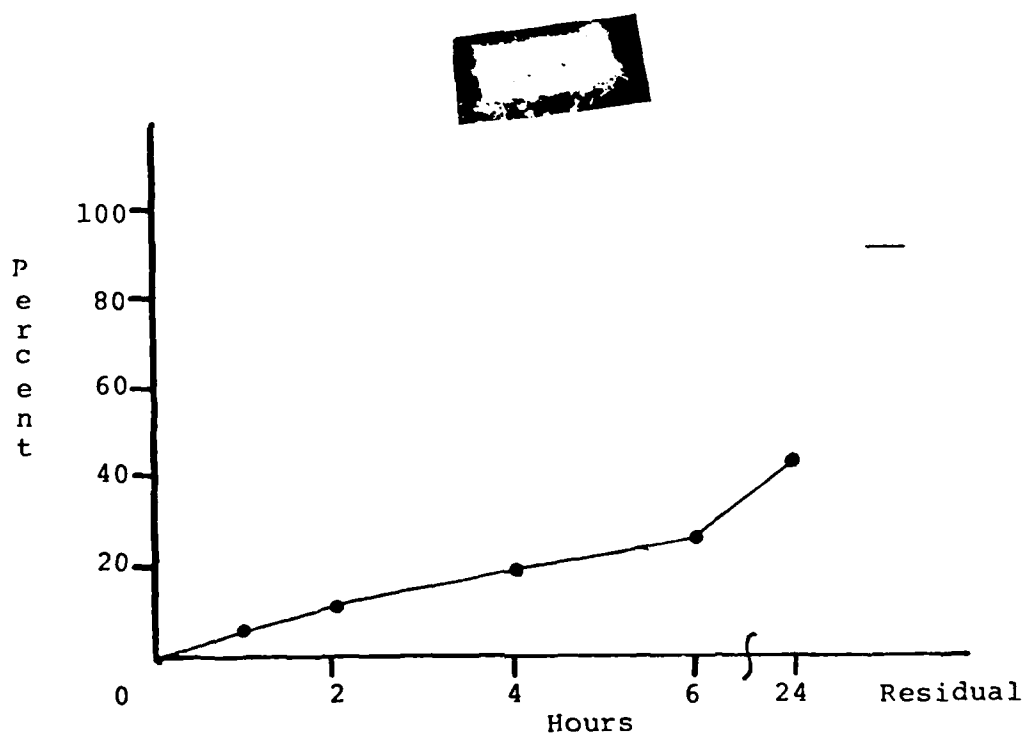
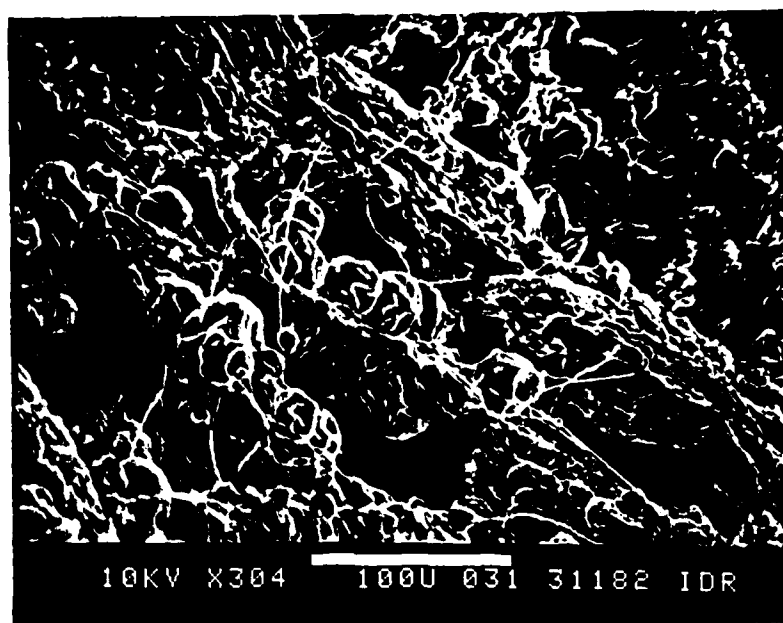


Figure 5 SEM, Sample, and Release Data for 20% Benzocaine Fabric

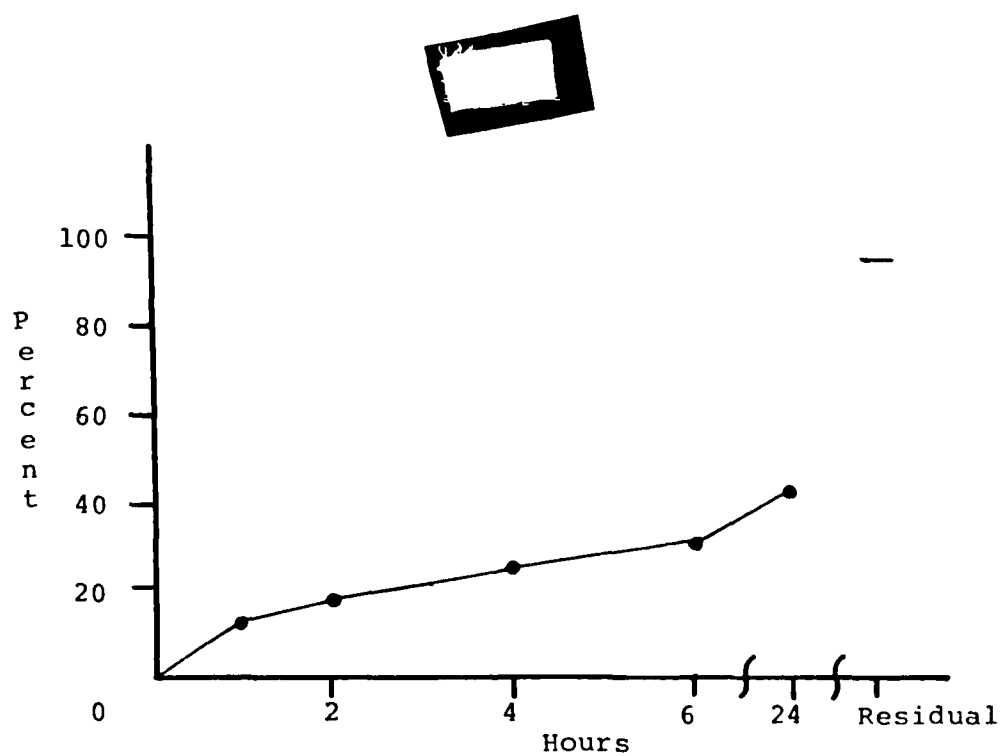
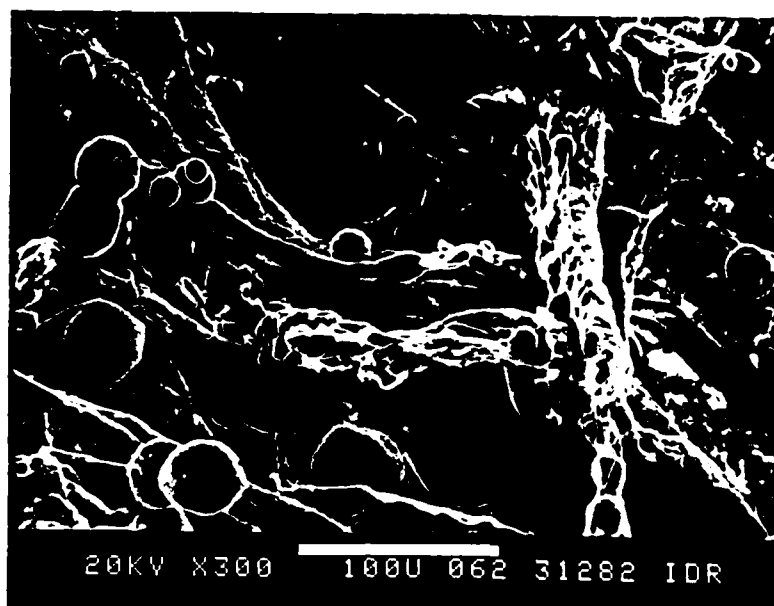


Figure 6 SEM, Sample, and Release Data for  
15% Etidocaine-HCl Fabric

trends of slower release for larger particles and particles with lower drug loading, we believe that the release is diffusion controlled.

Lidocaine·HCl powders release most of their available drug too rapidly for a sustained release wound dressing. However, large particles (300-425 microns) of 5% drug show some sustained release. Lidocaine (base) release is more sustained, as is benzocaine and etidocaine·HCl. In each case the larger particles release more favorably. Bupivacaine·HCl, at 10 and 17%, is released too rapidly, using this test system as a model of a wound dressing. SEMs, samples, and drug release data for selected lidocaine (base) and etidocaine·HCl powders are shown in Figures 7 and 8.

#### 5. Bupivacaine Comparisons

SEMs of 20% bupivacaine·HCl in fiber and powder form are shown in Figure 9. These materials have poor drug release characteristics (Figure 10). The release characteristics of similar 20% etidocaine·HCl fabric and powder are shown in addition to the bupivacaine data (Figure 10). If any conclusion can be drawn, the bupivacaine·HCl fabric is too fibrous, indicating a polymeric surface from which drug cannot diffuse (compare to the PVP·I<sub>2</sub> fabrics).

Bupivacaine·HCl fabric with 20% drug loading was analyzed by EDAX at two fiber nodules and one elongated fiber location. Values (11.1, 10.0, 11.6 c/s) indicated about 22% exposed drug and no drug segregation in this fabric. Unfortunately, drug release in vitro was 8% within one hour and only 3% additional release in 24 hours.

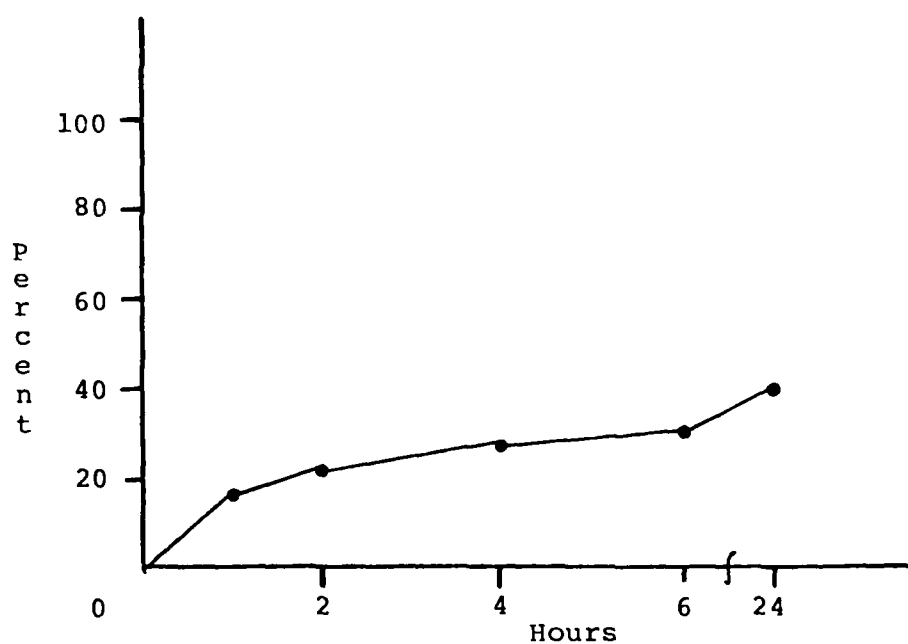
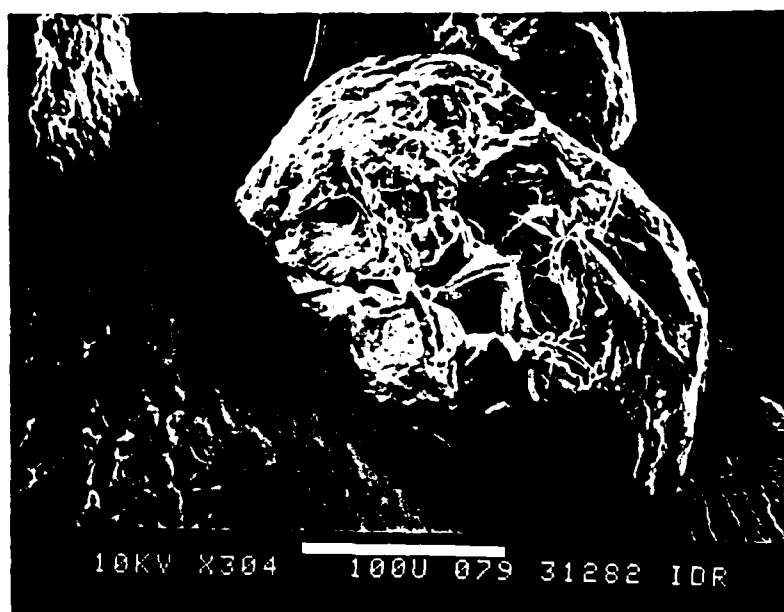


Figure 7 SEM, Sample, and Release Data for  
10% Lidocaine (Base) Powder of  
300-425 microns

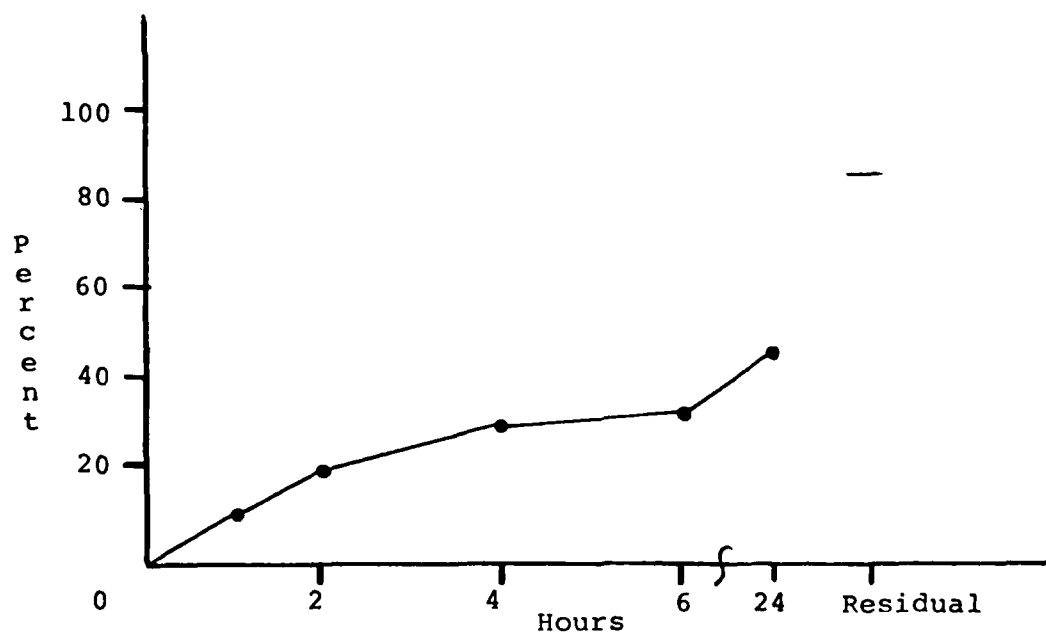
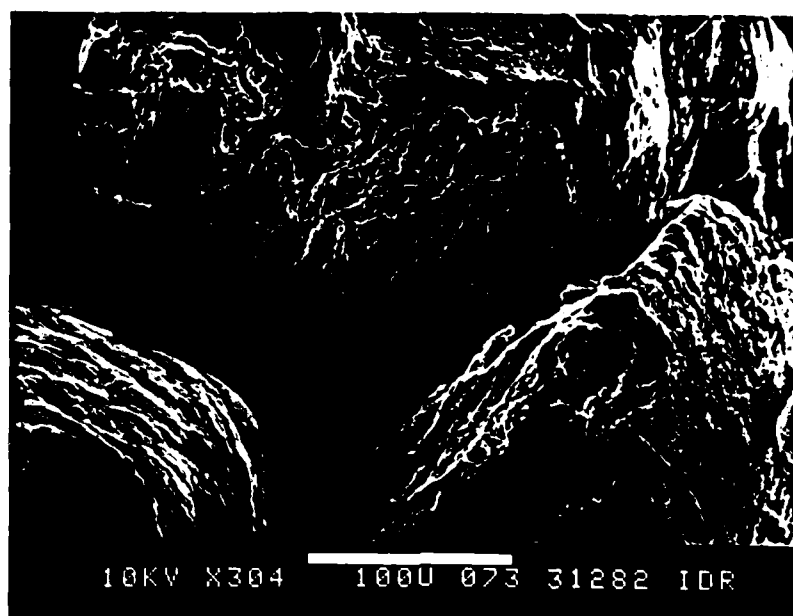
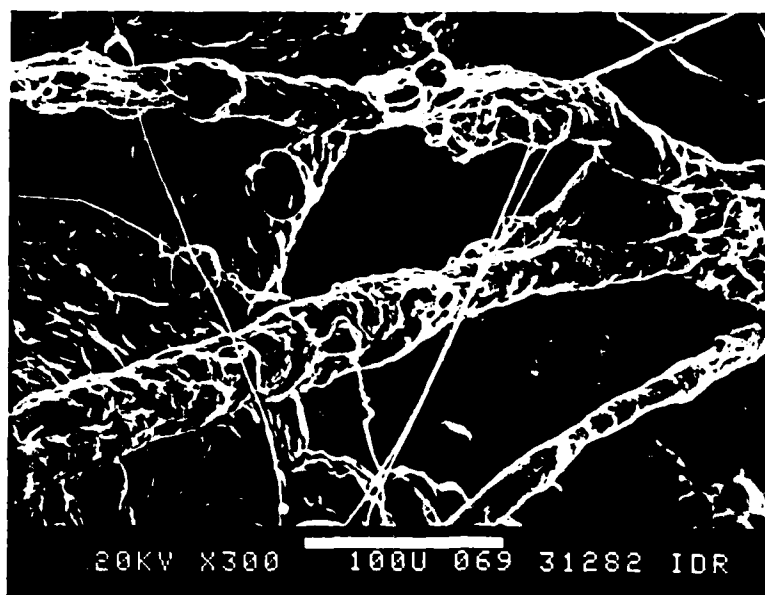


Figure 8 SEM, Sample, and Release Data for  
15% Etidocaine-HCl Powder of 300-425 microns



Fabric

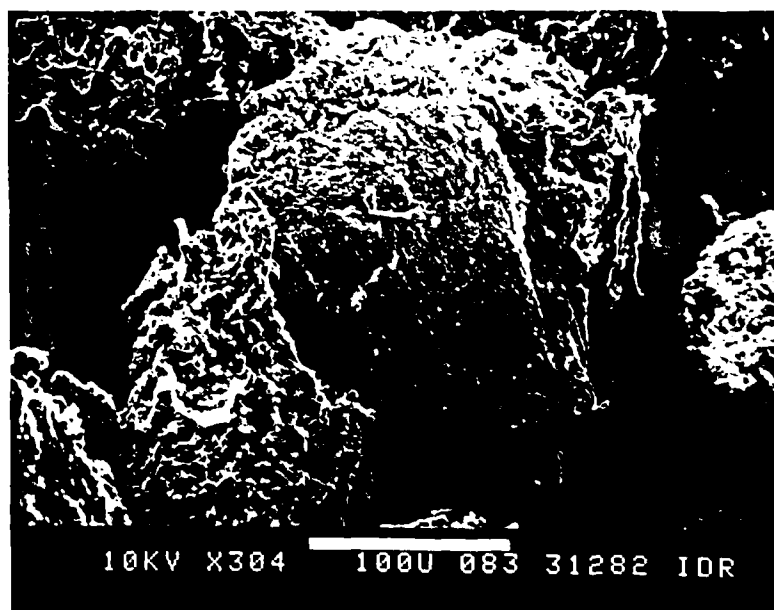
Powder  
(150-212 microns)

Figure 9 Bupivacaine·HCl Fabric and Powder  
at 20% Drug Loading

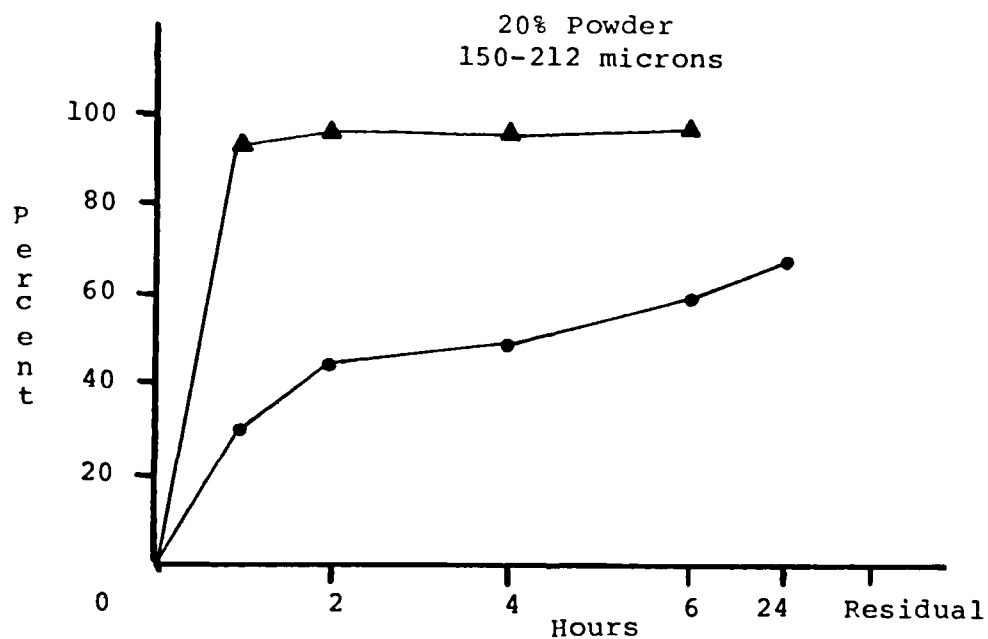
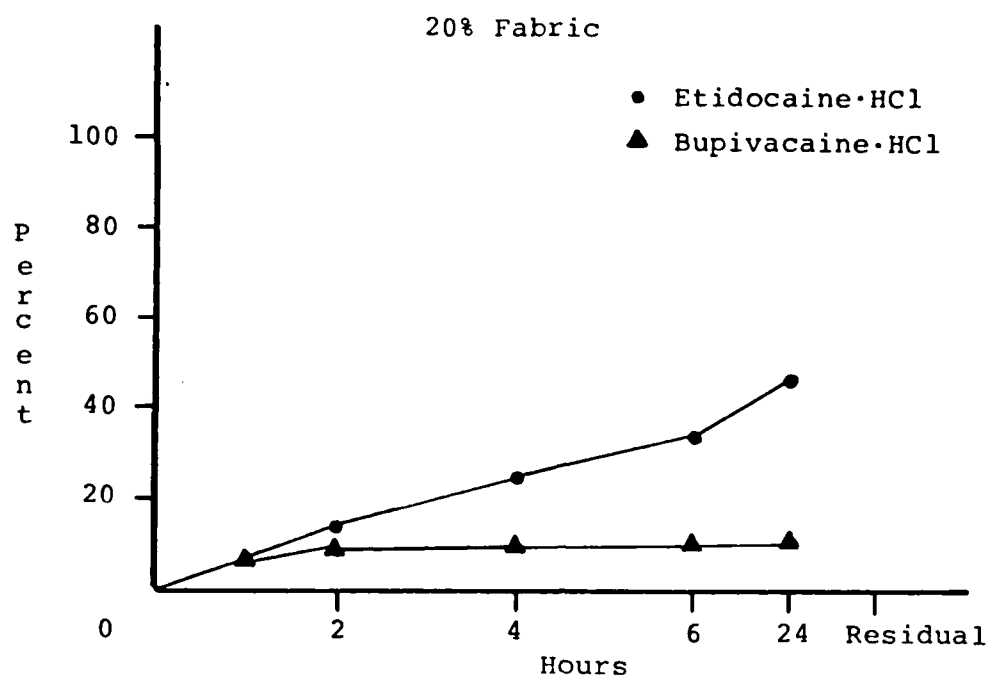


Figure 10 Comparison of Etidocaine·HCl and Bupivacaine·HCl at 20% Loading in Fabric and Powder 150-212 microns

Bupivacaine·HCl powder (150-212 microns) with 20% drug loading was similarly analyzed by EDAX. This sample was less homogeneous. Smooth surfaces had about 13% exposed drug, whereas rough surfaces had 26 and 41% exposed drug. It was noted that areas that were prone to electrostatic charging on the SEM had high drug (Cl) counts. Bupivacaine·HCl powder samples released almost all of the drug immediately (~90% in 1 hour). This size fraction was chosen for comparison to microcapsules on Contract No. DAMD17-81-C-1195.

## 6. General Conclusions on Anesthetic Release

General conclusions are that the base form of the anesthetics usually deliver drug in a more continuous manner than the salt forms, in both powder and fabric systems. Thus benzocaine release is relatively continuous, and lidocaine (base) release is more continuous than lidocaine·HCl or lidocaine·H<sub>2</sub>SO<sub>4</sub> release. Etidocaine·HCl release is better than bupivacaine·HCl release in both powder and fabric systems.

Theoretically, if 20% of the drug is released in one hour and the Higuchi model applies ( $M_t/M_\infty = kt^{1/2}$ ), then 49% should be released in 6 hours and almost all the drug should be released within 24 hours. Practically, much of the drug is unavailable for release in one day. However, when the entire sample is dissolved, all of the residual drug is found in the sample.

## 7. Storage Stability Testing

Selected powder and fabric samples have been stored under conditions described in Report No. 1 of DAMD17-80-C-0093. The samples which are stored and the conditions are summarized



in Table 6. The percent drug values are the nominal values, based on the drug to polymer ratio in the preparative solution.

Two additional samples were added recently because they are significantly different than the previously stored samples, and show more promising drug release behavior. These are a lidocaine (base) powder and a povidone-iodine fabric.

Four samples have been stored for one year and portions of these samples were analysed for drug content and release. The data, shown in Table 7, indicate that benzocaine cannot be stored at high temperature without hermetic sealing the sample.

TABLE 6  
SAMPLES STORED FOR FUTURE ANALYSIS

<u>SAMPLES STORED</u>		
		<u>Storage Date</u>
Powders		
20% Lidocaine·HCl	212-300 $\mu$ m	4/24/81
20% Etidocaine·HCl	212-300 $\mu$ m	4/24/81
20% Benzocaine	212-300 $\mu$ m	4/24/81
20% PVP-I <sub>2</sub> (Napp)	212-300 $\mu$ m	4/24/81
20% PVP-I <sub>2</sub> (BASF 17/12)	300-425 $\mu$ m	7/10/81
10% Lidocaine·base	300-425 $\mu$ m	5/28/82
Fabrics		
20% Lidocaine (base)		7/10/81
20% Etidocaine·HCl		7/10/81
20% Benzocaine		7/10/81
20% PVP-I <sub>2</sub> (BASF 17/12)		7/14/81
40% PVP-I <sub>2</sub> (BASF 17/12)		5/28/82

STORAGE CONDITIONS

<u>Condition</u>	<u>Temperature</u>	<u>Humidity</u>	<u>Light</u>
1	40°C	Ambient	None
2	40°C	Desiccate	None
3	Ambient	Ambient	None
4	Ambient	Ambient	Ambient
5	4°C	Ambient	None
6	4°C	Desiccate	None

TABLE 7  
ASSAY AND DRUG RELEASE OF POWDERS AFTER ONE YEAR OF STORAGE

Sample	Hours of Release	Original Data	One Year Later			
			40° C		R.T.	
			Dark	Sealed	Dark	Sealed
Etidocaine· HCl, 20% 212-300 µm	1	44	31	-	52	40
	6	59	56	-	55	57
	24	75	56	-	61	60
	Assay %	-	24	-	24	24
Lidocaine· HCl, 20% 212-300 µm	1	68	76	-	82	76
	6	78	78	-	84	76
	24	80	80	-	86	78
	Assay %	-	23	-	22	23
Benzocaine 20% drug 212-300 µm	1	34	0	40	18	22
	6	56	1	54	34	38
	24	66	5	62	46	51
	Assay %	19	8	20	19	17
PVP-I <sub>2</sub> , Napp 20% drug 212-300 µm	1	24	46	-	40	-
	6	24	69	-	53	-
	24	39	91	-	75	-
	Assay %	-	12	-	12	-

## B. Biological Testing

### 1. Povidone-Iodine Evaluation

Povidone iodine releases iodine as required to maintain an iodine concentration sufficient to oxidize amino acids of the proteins of bacteria, fungi, etc. (Schelzinger, Mahinka and Levin, 1979). This available iodine will also react with local tissue protein. However, systemic metabolism of the byproducts occurs long after the oxidizing activity of PVP·I<sub>2</sub> is spent. Hence many of the antiseptic properties of the slow release PVP·I<sub>2</sub> preparations can be assessed by bacterial culture techniques, without involving a live animal.

Bacillus subtilis was used as the test microorganism. This nonpathogenic bacteria was suggested by USAIDR personnel and was used by Lowbury, et al (1964) as a test microorganism in PVP·I<sub>2</sub> studies.

B. subtilis spores were obtained from Raven Biological Laboratories, Inc. Omaha, Nebraska (ATCC No. 9372, 10<sup>5</sup> spores/ml). Tryptic soy broth from Difco (Bacto, dehydrated, pH 7.3) was chosen as the culture medium. This was prepared at 30 g/l and sterilized in our diffusion cells by our consultant, Joel Ackerman. Cells were grown overnight in this medium and then aliquots were transferred to test cells which contained various antiseptic preparations.

In the first experiment 100 ml of broth was used in 250 ml erlenmeyer flasks. Overnight growth from 0.1 ml of spores leveled off at about 1.3 absorbance units, at 670 nm. Ten milliliters were used to inoculate the test flasks. Various quantities of PVP·I<sub>2</sub> (BASF 17/12) were tested, and 1.0 grams of drug (1%) was found to be bacteriostatic, whereas 0.3 grams (0.3%) was not. In the second experiment 37 ml of broth was used in the

standard diffusion cells. The normal inoculum was 3 ml of a 24-hour growth. The data of this experiment are shown in Table 8.

This experiment showed that 0.5% PVP·I<sub>2</sub> in this broth is bacteriostatic. This required 200 mg of PVP·I<sub>2</sub>. A concentration of 0.25% was not bacteriostatic, even with 1/3 the challenge of B. subtilis. Splitting the "standard" challenge into 3 equal increments, as in reinfection, was controlled by a single dose of 300 mg (0.75%) of PVP·I<sub>2</sub>. Splitting the drug into three equal doses, as for a time release drug, controlled the bacterial growth, although there was some early bacterial growth.

At this point the technique was refined. A microcell was purchased which requires only 1 ml of solution. This solution for analysis was decolorized with 10 µl of 130 mg/ml Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution. Finally drug preparations were maintained in diffusion bags (polyester screen) to aid in solution transfers.

In the next experiments B. subtilis was added at 0, 1, and 2 hours as in Tube 8 (Table 8). Pure PVP·I<sub>2</sub> was compared with the 40% PVP·I<sub>2</sub> in fabric which is described above. In the first of these experiments all of these tubes grew bacteria (Abs > 1.7 at 24 hours for 100, 150, 200, 250, 300 mg PVP·I<sub>2</sub> as drug pure or in fabric). There was an indication at four hours that the pure drug was more inhibitory to bacterial growth than the fabric containing the drug. In the next experiment 200, 300, 400 mg of PVP·I<sub>2</sub>, as pure drug and in fabric, was measured with and without diffusion bags. Blanks were run with and without bags, without B. subtilis. The results are shown in Table 9. A ranging experiment was also performed with B. subtilis spores (Tubes 11-13).

TABLE 8  
INHIBITION OF BACILLUS SUBTILIS GROWTH  
BY PURE PVP-I<sub>2</sub>

Tube No.	PVP-I <sub>2</sub> mg	B. Subtilis ml	Absorbance at Various Times				
			0	1	2	4	24 (Hrs.)
1	0	0	0.01	0.01	0.01	0.01	0.01
2	0	3	0.22	0.34	0.72	1.42	3.3
3	100	1	0.10	0.11	0.15	0.73	3.3
4	100	3	0.22	0.30	0.58	1.17	2.6
5	200	3	0.22	0.23	0.22	0.22	0.12
6	300	3	0.27*	0.22	0.21	0.22	0.17
7	100	3	0.22	0.30			
	+100 (1 hr)			0.30	0.30		
	+100 (2 hr)				0.32*	0.28	0.21
8	300	1	0.20*	0.14			
		+1 (1 hr)		0.22	0.21		
		+1 (2 hr)			0.31	0.29	0.20

\*distinct blue color of povidone iodine

TABLE 9  
B. SUBTILIS INHIBITION BY PVP·I<sub>2</sub> IN FABRIC

Tube	PVP·I <sub>2</sub> ** mg	PVP·I <sub>2</sub> (fabric) mg (PVP·I <sub>2</sub> )	Bag None	Cells* Spores	Absorbance of 670nm (Turbidity)		
					2 Hr.	4 Hr.	24 Hrs.
1	200	-	b	c	0.25	0.86	1.44
2	300	-	b	c	0.23	0.39	1.83
3	400	-	b	c	0.21	0.29	0.21
4	200	-	n	c	0.34	1.07	1.90
5	300	-	n	c	0.25	0.62	1.86
6	400	-	n	c	0.22	0.31	0.23
7	-	200	b	c	0.25	1.04	1.66
8	-	300	b	c	0.23	0.38	1.67
9	-	400	b	c	0.18	0.21	0.07
10	-	300	n	c	0.28	0.45	1.82
11	30	-	b	s	0.02	0.02	1.80
12	100	-	b	s	0.04	0.04	1.78
13	300	-	b	s	0.06	0.06	0.05
B <sub>1</sub>	-	-	b	-	0.02	0.02	>2.0
B <sub>2</sub>	-	-	n	-	0.01	0.01	0.02

\* 1 ml of 24 growth cells added at 0, 1, 2 hours

\*\* PVP·I<sub>2</sub> added at t=0 to 39 ml of TS Broth

In this experiment it is evident that 300 mg PVP·I<sub>2</sub> (per 40 ml broth) is no longer bacteriostatic to three challenges of 1 ml of the 24-growth medium; but 400 mg is bacteriostatic. With 300 mg of PVP·I<sub>2</sub> as drug or fabric the inhibition at 4 hours is similar (0.39 and 0.62 versus 0.38 and 0.45). The blanks indicate that handling of the bags (and samples) causes insterility producing a heavy microbial growth within 24 hours. However, we believe this is not significant with respect to the challenge of B. subtilis cell inoculation.

Thus in this experiment there was no proven advantage (nor disadvantage) to the use of PVP·I<sub>2</sub> slow release fabric. The fabric is easy to apply and may cause less of a toxic or burning effect than the same amount of PVP·I<sub>2</sub> in solution. Alternately the slow release might be more effective in a test system in which there is limited access to fluid and in which there is an infinite sink for the active iodine.

## 2. Avitene Evaluation

The hemostatic evaluation of Avitene was proposed using a uniformly bleeding wound generated by a dermatome. The method of Wilkinson, Tenery and Zuffi (1973) was modified for use on a rabbit.

After a discussion with Dennis Orgill at MIT, a Goulian skin graft knife was selected and purchased from E. Weck Co. (Research Triangle Park, N.C.).



In a preliminary experiment, one rabbit was anesthetized with ketamine and a bleeding surface prepared, under the direction of Mr. Orgill, using a 0.012 inch blade guide. The rabbit skin is considerably thinner than the corresponding skin of a human or guinea pig. Although our technique did not yield a usable skin graft, it yielded a relatively uniform bleeding surface.

One surface was blotted with a sponge and then covered for 5 minutes with a sectional dressing of Avitene and cotton (U.S.P.). The non-sterile dressing was applied without a gauze covering, but was then replaced by a standard sterile dressing after 5 minutes. The blood on the sectional dressing was analysed using a commercial cyanmethemoglobin reagent (Drabkin, Baker Diagnostics) and compared to the assay using rabbit blood drawn with an anticoagulant. For the single preliminary experiment approximately 16  $\mu$ l of blood was absorbed into Avitene and 62  $\mu$ l absorbed into cotton.

In more recent experiments we have obtained more professional skin grafts, but have obtained even less blood at the donor site. More bleeding was attempted by warming the area with a heat lamp, using less ketamine, and using lidocaine (vasodilator) as a topical anesthetic. If necessary, we will inject heparin or an  $\alpha$ -blocker to obtain a uniform bleeding surface which will continue to ooze blood.

Dried blood on Avitene and cotton is difficult to remove, and a stain remains after 5 minutes immersion in the cyanomethemoglobin reagent. Experiments were therefore performed with known volumes (20  $\mu$ l) of freshly drawn blood, placed directly in the reagent and after storage on Avitene and cotton. The reagent color is stable for at least one day after addition of blood. With dried blood, a long reaction time (hours to days) is required to obtain the correct absorbance. However, if the sample is placed in the reagent 5 to 7 minutes after collection on the dressing, then the correct absorbance (0.30-0.35) is obtained by the time the sample is reacted for 5 minutes and the fibrous dressing is centrifuged down to the bottom of the tube. The fibrous mat does not have a residual blood stain. Therefore, this method will be used to handle blood samples obtained in the controlled bleeding experiments.

III. REFERENCES

- Baker, R.W., Lonsdale, H.K. (1974) "Controlled Release: Mechanism and Rates" in Controlled Release of Biologically Active Agents, p. 51
- Flory, P.J. (1953) "Principles of Polymer Chemistry", Cornell Univ. Press, Ithica, NY, p. 325.
- Lowbury, E.J.L., Lilly, H.A., Bull, J.P. (1964) Brit. Med. J. 2, 531-536.
- Schenck, H-U, Simak, P., Haedicke, F. (1979) J. Pharm Sci., 68, 1505-1509.
- Schlezinger, J., Mahinka, S.P., Levin, B.A. (1979) Comments of BASF AG Regarding Povidone-Iodine, Vol. 1 before FDA in the matter of the Proposed Monograph for Antimicrobial Products.
- Wilkinson, T.S., Tenery, J.H., Zufi, D. (1973) Plastic and Reconstructive Surgery, 51, 541-544.

## DISTRIBUTION LIST

4 copies

Commander  
US Army Medical Research and Development Command  
ATTN: SGRD-RMS  
Fort Detrick, Frederick, Maryland 21701-5012

12 copies

Defense Technical Information Center (DTIC)  
ATTN: DTIC-DDAC  
Cameron Station  
Alexandria, VA 22304-6145

1 copy

Dean  
School of Medicine  
Uniformed Services University of the  
Health Sciences  
4301 Jones Bridge Road  
Bethesda, MD 20814-4799

1 copy

Commandant  
Academy of Health Sciences, US Army  
ATTN: AHS-CDM  
Fort Sam Houston, TX 78234-6100

END

DATE  
FILMED

4-85

DTIC